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PTO/SB/05 (4/98)

**UTILITY
PATENT APPLICATION
TRANSMITTAL**

(Only for new nonprovisional applications under 37 C.F.R. § 1.53(b))

Attorney Docket No. 571-651

First Inventor or Application Identifier Lisa Cousins

Title ANALYSIS TECHNIQUE, INCORPORATING SELECTIVE

Express Mail Label No.

APPLICATION ELEMENTS

See MPFP chapter 600 concerning utility patent application contents.

1. ☐ * Fee Transmittal Form (e.g., PTO/SB/17)
(Submit an original and a duplicate for fee processing)
2. ☒ Specification (Total Pages **33**)
(preferred arrangement set forth below)
 - Descriptive title of the invention
 - Cross References to Related Applications
 - Statement Regarding Fed sponsored R & D
 - Reference to Microfiche Appendix
 - Background of the invention
 - Brief Summary of the invention
 - Brief Description of the Drawings (if filed)
 - Detailed Description
 - Claim(s)
 - Abstract of the Disclosure
3. ☒ Drawing(s) (35 U.S.C. 113) (Total Sheets **8**)
4. Oath or Declaration (Total Pages **1**)
 - a. ☐ Newly executed (original or copy)
 - b. ☐ Copy from a prior application (37 C.F.R. § 1.63(d))
(for continuation/divisional with Box 16 completed)
 - i. ☐ **DELETION OF INVENTOR(S)**
Signed statement attached deleting inventor(s) named in the prior application, see 37 C.F.R. §§ 1.63(d)(2) and 1.33(b).

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5. ☐ Microfiche Computer Program (Appendix)
6. Nucleotide and/or Amino Acid Sequence Submission
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 - a. ☐ Computer Readable Copy
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ACCOMPANYING APPLICATION PARTS

7. ☐ Assignment Papers (cover sheet & document(s))
8. ☐ 37 C.F.R. § 3.73(b) Statement ☐ Power of Attorney
(when there is an assignee)
9. ☐ English Translation Document (if applicable)
10. ☐ Information Disclosure Statement (IDS)/PTO-1449 ☐ Copies of IDS Citations
11. ☐ Preliminary Amendment
12. ☐ Return Receipt Postcard (MPEP 603)
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UNITED STATES

Inventors: Lisa Cousins, Bruce Thomson

- 1 -

**Title: ANALYSIS TECHNIQUE, INCORPORATING SELECTIVELY
INDUCED COLLISION DISSOCIATION AND SUBTRACTION OF
SPECTRA**

FIELD OF THE INVENTION

5 This invention relates mass spectrometers, and more particularly is concerned with collision-induced dissociation (CID) in a tandem mass spectrometer. The invention is particularly intended to enable multiple stages of fragmentation, and hence mass analysis or spectroscopy, to be effected in a collision cell.

10 **BACKGROUND OF THE INVENTION**

 Radio frequency (RF) only multipole spectrometers, more particularly quadrupole spectrometers, are widely applied in mass spectrometry and nuclear physics, due to their ability to transport ions with minimal losses. During such transportation of the ions, the initial ion
15 positions and velocities change, but the total phase space volume occupied by the ion beam remains constant (see Dawson, P.H., "Quadrupole Mass Spectrometry and its Applications," Elsevier Scientific Publishing Co., New York, 1976). However, if a buffer gas is introduced into the ion guide, a dissipative process occurs, due to ion molecule collisions, and this enables
20 an ion beam to be focused onto the quadrupole axis after the initial velocities have been damped.

 Collisional quadrupole or other multipole devices have been used as an ion guide providing an interface between an ion source and a mass spectrometer, or alternatively as a collision cell for collision-induced
25 dissociation (CID) experiments. As a straightforward interface, collisional damping reduces the space and velocity distributions of the ions leaving the ion source, thus improving the beam quality. For CID experiments, primary ions having relatively large velocities enter the multipole and collide with buffer gas molecules, and so collision-induced dissociation takes place. The
30 multipole helps to keep both primary ions and fragment ions, resulting from the collision-induced dissociation, close to the axis and to deliver

them to the exit for further analysis. Collisions inside the multipole spectrometer again act to reduce the space and velocity distribution of the ion beam.

Ion motion in a perfect quadrupole field is governed by Mathieu's equation (See Dawson as cited above); ions oscillate around the quadrupole axis at an appropriate fundamental frequency which is determined by their m/z and quadrupole parameters, and is independent of ion position and velocity. If the frequency of any periodic forces acting on ions coincides with the ion fundamental frequency, then resonance excitation takes place. Similar resonance excitation is widely applied in quadrupole ion trap or in ion cyclotron resonance mass spectrometers (R.E. March, R.J. Hughes, "Quadrupole storage mass spectrometry," 1989, John Wiley & Sons).

These properties of spectrometers have been employed in many ways. Thus, in U.S. provisional patent application 60/046,926 filed May 16, 1997 (and related U.S. patent application 09/066,556 and Canadian patent application 2,236,199), there is disclosed a high pressure MS-MS system. This was intended to provide improvements to a conventional triple quadrupole mass spectrometer arrangement, employing two precision quadrupole mass spectrometers separated by an RF-only quadrupole which is operated as a gas collision cell. The first mass spectrometer is used to select a specific ion mass-to-charge ratio (m/z), and to transmit the selected ions into the RF-only quadrupole or collision cell. In the RF-only quadrupole collision cell, some or all of the parent ions are fragmented by collisions with the background gas, commonly argon or nitrogen, at a pressure of up to several millitorr. The fragment ions, along with any unfragmented parent ions are then transmitted into the second precision-quadrupole which is operated in a mass resolving mode. Usually, the mass resolving mode of this second spectrometer is set to scan over a specified mass range, or else to transmit selected ion fragments by peak hopping, i.e. by being rapidly adjusted to select specific ion m/z ratios in sequence. The ions transmitted through this spectrometer are detected by an ion detector.

- 3 -

A problem with this conventional arrangement is that the two mass resolving quadrupoles are required to operate in the high vacuum region (less than 10^{-5} torr), while the intermediate collision cell operates at a pressure up to several millitorr. That earlier invention was intended to
5 simplify the apparatus and eliminate the necessity for separate RF-only and resolving spectrometers at the input to the apparatus. Instead, a single quadrupole is provided, operating in the RF-mode to act as a high pass filter. Additionally, this quadrupole is provided with an AC field, which can be identified as a "filtered noise field", which contains a notch in the
10 frequency range corresponding to the mass of an ion of interest. This notch can be moved, to select and separate desired ions.

Other older proposals can be found, for example, in U.S. Patent 5,420,425 (Bier et al. and assigned to Finnigan Corporation). This relates to an ion trap mass spectrometer, for analyzing ions. It has electrodes
15 shaped to promote an enlarged ion occupied volume. A quadrupole field is provided to trap ions within a predetermined range of mass to charge ratios. Then, the quadrupole field is changed so that trapped ions with specific masses become unstable and leave the trapping chamber in a direction orthogonal to the central axis of the chamber. The ions leaving the
20 spectrometer are detected, to provide a signal indicative of their mass-to-charge ratios. One method that is taught in this patent is to first introduce ions within a predetermined range of mass-to-charge ratios into the chamber and subsequently to change the field to select just some ions for further manipulation. The quadrupole field is then adjusted so as to be
25 capable of trapping product ions of the remaining ions, and the remaining ions are then dissociated or reacted with a neutral gas to form those product ions. Subsequently, the quadrupole field is changed again, to remove, for detection, ions whose mass-to-charge ratios lie within the desired range, which ions are then detected.

30 The above process describes how the technique of MS/MS (or MS^2) is applied in an ion trap configuration. A related technique of MS/MS/MS (or MS^3) can be provided by isolating one of the product ions of

- 4 -

the first MS² process, and eliminating all but the selected product ion from the trap. The selected product ion mass is then excited so that it fragments through collisions with the buffer gas in the trap. The range of secondary product ions formed in this two-stage process is then scanned from the trap
5 for detection, so that a mass spectrum is recorded. The spectrum consists of fragments of a fragment from the original parent ion. The process can be extended by trapping and isolating one of the secondary product ions, and then fragmenting that ion mass, in order to form an MS/MS/MS/MS or MS⁴ spectrum, and ultimately the process can be extended to an MSⁿ
10 spectrum. Ion losses occur at each stage, however, so that sensitivity decreases as the number of steps increases. Nevertheless, this technique of MSⁿ can be a useful tool to help elucidate the structure of organic ions.

Another approach to obtaining an MS³ spectrum is described in U.S. Patent 6,011,259 by Craig Whitehouse, Thomas Dresch and
15 Bruce Andrien of Analytica of Brantford, which shows how a multipole ion guide can be combined with a time-of-flight (TOF) mass spectrometer to provide MSⁿ analysis. They describe the method of using resonant excitation to excite one ion mass in the quadrupole ion guide in order to fragment a selected ion (without isolating or rejecting the other ion masses).
20 By turning the excitation on and off several times per second, a background subtracted spectrum of the fragments of the desired precursor ion can be created. This works very well with TOF mass spectrometers where the TOF is pulsed at a higher frequency than the pulsing of the excitation.

The method described produces an MS² spectrum of the
25 selected ion. In order to produce an MS³ spectrum (designated by the inventors of that patent as MS/MS²), two frequencies must be added, first exciting just the first precursor ion, then adding another frequency to excite both the first precursor ion and the selected product of that precursor ion together, and subtracting the spectra to obtain an MS³ spectrum. A total of
30 three spectra must be collected sequentially: a first spectrum without any excitation, then a spectrum with only one excitation frequency (first MS² spectrum), then a spectrum with both frequencies added simultaneously

- 5 -

(MS³). The second spectrum must be subtracted from the first spectrum in order to generate the MS² spectrum and identify the primary product ion of interest, and then the third spectrum must be subtracted from the second spectrum in order to generate an MS³ spectrum (in other words, an MS² spectrum of the primary product ion).

The first technique taught above is complex, and requires a number of separate quadrupoles or the like, and the ability to move the ions sequentially through the different quadrupole sections. The technique taught in the Finnigan patent is complex and requires a number of steps. Also, it is concerned with ion traps and not a flow quadrupole. While all of the above methods can be used to obtain MS³ spectra (or higher order), they all suffer from some limitations or drawbacks. The ion trapping methods require isolation of the first parent ions before fragmentation, and then sequential steps of fragmentation, isolation and fragmentation in order to reach MS³. The initial ion mass which is fragmented is not mass selected.

The method described in Whitehouse et al to achieve MS³ is complex, and reduces the duty cycle for the overall process (as described by the inventors of the '259 patent) to 33% for MS³. Also, the mass-selective specificity of the first fragmentation step, obtained by exciting the ion radially to collide and fragment is much less than that achievable by using a mass spectrometer (a quadrupole mass filter for example). Therefore, the method taught is both less sensitive and less mass-selective than that described in the present application. Finally, these inventors fail to recognize that simple subtraction alone will not always give an accurate representation of the true fragment spectra; the present inventors have realized further statistical analysis is required to eliminate uncertainties due to poorly subtracted spectra.

Accordingly, it is desirable to provide one technique which, in one device, readily enables ions of a selected mass-to-charge ratio to be subject to collision-induced-dissociation (CID) or fragmentation, so that the fragments can be transported further for subsequent analysis. It is desirable to provide this in a single device, since movement of ions from one device

- 6 -

to another inevitably leads to some losses. Similarly, the techniques of the Finnigan patent works effectively with pulse ion sources, but inefficiently with continuous ion flow, for instance from an electrospray ion source. In this field, spectrometers are frequently used to analyze small samples, and often, high efficiency is required, if any reliable reading or measurement is to be obtained.

SUMMARY OF THE INVENTION

In accordance with a first aspect of the present invention, there is provided a method of analyzing a substance, the method consisting of:

- 10 (1) creating a stream of ions in said substance;
- (2) supplying the stream of ions and a collision gas to a multipole and providing an RF signal to the multipole, whereby the quadrupole functions as a collision cell;
- (3) fragmenting said ions in the RF multipole by collisions
15 with the gas molecules, in order to form primary fragment ions;
- (4) supplying additional alternating current to the multipole at a frequency selected to cause resonance excitation of a desired primary fragment ion mass-to-charge ratio, whereby ions with said desired primary fragment ion mass to charge ratio are excited and undergo collisions with
20 the gas molecules causing production of secondary fragment ions;
- (5) modulating the alternating current signal applied in step (4) whereby periods in which said alternating current signal is applied alternate with periods in which the alternating signal is not applied;
- (6) detecting the ion signal after fragmentation with a mass
25 spectrometer and collecting one set of data for one spectrum, representative of the ion spectrum when the alternating current signal is applied and another set of data for another spectrum, representative of the ion spectrum when the alternating current signal is not applied;
- whereby said other spectrum can be subtracted from said one spectrum, to
30 generate a subtracted spectrum showing the secondary fragment ions without the presence of the primary fragment ions except for any said

- 7 -

primary fragment ions which are generated by step (4), whereby to obtain MS³ information.

In one embodiment, the method further includes the step of processing the data sets by applying statistical analysis to reject spectra
5 having statistically insignificant variations in the ion signal.

Preferably, the statistical analysis is implemented in a software program and performed automatically.

More preferably, the statistical analysis is performed in real time so that spectra having statistically insignificant variations in the ion
10 signal are not displayed.

In one embodiment, the alternating current signal at a frequency that excites the desired primary fragment ion.

Preferably, the method includes passing the stream of ions through a first mass analyzer to select a precursor ion of interest, and
15 passing the precursor ion into the collision cell.

More preferably, the method includes providing a potential difference between the first mass analyzer and the collision cell, to accelerate the precursor ion into the collision cell, whereby the precursor ions gain sufficient velocity to collide with the buffer gas to cause fragmentation, and
20 wherein step (4) comprises applying an alternating current signal to excite a fragment of the precursor ion, said fragment comprising the desired ion.

The method can include applying a second alternating current signal to the quadrupole rod set, to excite a fragment ion resulting from resonance excitation of said desired ion, thereby to generate secondary
25 fragment ions and wherein step (5) comprises modulating the second alternating current signal. It will be appreciated that it may be possible to apply a number of different excitation signals to cause fragmentation of fragments from the previous step.

Another aspect of the present invention provides an
30 apparatus, for analyzing a substance by resonance excitation of selected ions and selective collision-induced dissociation, the apparatus comprising:

an ion source for generating a stream of ions;

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Preferably the apparatus includes a first mass analysis section
25 for selecting a parent ion and a final mass analysis section, including the
detector, for analyzing fragment ions from the collision cell.

Figure 1 is a schematic of a first embodiment of an apparatus

- 9 -

in accordance with the present invention;

Figure 2 is a schematic of an apparatus in accordance with a second embodiment of the present invention;

Figures 3a-3e are mass spectra showing analysis of bosentan
5 and fragments thereof;

Figures 4a, 4b and 4c are spectra showing fragmentation of taxol;

Figures 5a and 5b are detailed graphical spectra of fragments obtained from fragmentation of a fragment of mass 202 of bosentan;

10 Figures 6a-6c and 7a-7c are mass spectra showing MS³ and MS⁴ fragmentation schemes for reserpine; and

Figures 8a-8d are subtracted MS³ mass spectra of Reserpine at various excitation amplitudes.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

15 A description is first given of the apparatus in Figures 1 and 2. The two apparatus are largely similar, except for the final mass analysis stage. Figure 1 shows a variant with a quadrupole rod set and detector as the final mass analysis stage, while this is effected by a time-of-flight section in Figure 2.

20 Referring first to Figure 1, the first variant of the apparatus is indicated at 10. In known manner, the apparatus 10 includes a first quadrupole rod set generally indicated as Q0. Q0 is intended to collimate and reduce the energy of ions received from an electrospray source or the like. In known manner, upstream of Q0, there would be an ion inlet,
25 skimmers, intermediate pressure stages and the like, all intended to remove gas and reduce pressure down to that required for mass analysis (these elements and associated pumps are not shown). Q0 collimates the ion beam and further serves to reduce gas pressure.

Ions from Q0 pass through an interquad aperture 12 into a
30 quadrupole rod set Q1, which functions as a first mass analysis section. In known manner, Q1 is supplied with resolving RF and DC voltages. These

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- 10 -

can be conventional and the power supplies are not shown.

From Q1, the ions pass through into a collision cell housed in a chamber generally indicated 14. The collision cell includes a quadrupole rod set Q2. The chamber 14 includes, at either end, an inlet interquad aperture 16 and an exit interquad aperture 18.

The ions then pass into a final quadrupole Q3. Q3 again would be provided with resolving RF and DC voltages, and the power supply for these is not shown. Finally, the ions pass through to a detector 20.

In known manner, appropriate DC potentials would be provided between the different quadrupole sections Q0, Q1, Q2 and Q3 and also appropriate potentials on the interquad apertures 12, 16, 18, together with an appropriate potential drop to the detector 20. These various potentials ensure movement of ions axially, from left to right in Figure 1, in known manner.

Quadrupoles Q1, Q3 would be maintained at a low pressure of 10^{-5} torr, as is known for mass resolving quadrupoles. Chamber 14 is operated as a collision cell and would be provided with a suitable collision gas (source not shown). Typically, it is operated at a pressure in the range 0.5-20 mTorr. A suitable collision gas is nitrogen.

In accordance with the present invention, a first MS step is effected in Q1. This selects a parent or precursor ion, which then passes into the rod set Q2 of the collision cell. To effect a second MS step, MS^2 , ions are accelerated into a quadrupole collision cell (Q2), effecting fragmentation through collision with a low pressure gas in Q2 to generate primary fragment ions. A radio frequency (RF) source 22 for rod set Q2 is indicated, for example, 1,000 volts at 2 MHz. An auxiliary RF voltage would be provided in a quadrupolar, dipolar or any other suitable manner, i.e. with the $\cos \omega t$ provided to one opposite pair of rods in the quadrupole rod set Q2, and $-\cos \omega t$ provided to the other, diagonally opposite pair of rods of the rod set Q2.

It will be appreciated that while each fragmentation step is

- 11 -

designated MS^2 , MS^3 , etc., the final MS step is effected in Q2 (or other downstream mass analyzer). Also, the number of fragmented ion steps is 1 less than the total number of MS steps, i.e. MS^2 has one fragmentation step, MS^3 has two fragmentation steps, and MS^n has $n-1$ fragmentation steps.

5 Another way of expressing this is to note that a mass analysis step as such is not effected in the collision cell or quadrupole Q2. Rather fragment ions are generated in Q2 for mass-analysis in a downstream mass analyzer. Thus a reference, for example, to MS^2 occurring in Q2 means that fragment ions are generated in Q2, and that these are then mass analyzed
10 downstream in Q3, to provide the second mass analysis step.

Note also, while this description of the preferred embodiment assumes in all cases a first mass analysis step in Q1, this may not be essential. Thus, it may be desirable to analyze ions from a pure, single component sample, if only to record the characteristic fragmentation
15 characteristics of the chosen component. In such a case, it may be possible to omit the first mass selection step in Q1. In this case, the total number of fragmentation steps will equal the number of mass-analysis steps.

Now, in accordance with the present invention, the rod set Q2 is further excited to effect either one or a multiple steps of excitation.

20 Firstly, a further excitation step MS^3 is effected by an excitation source 24 provided with a modulation control unit 26, whose function is explained below which causes secondary fragment ions to be generated from the primary fragment ions. To effect a third or tertiary fragmentation step, a second power supply 28 is provided, connected to a second modulation
25 control unit 30. Each of the power supplies 24, 28 can provide a similar signal to the rod set Q2, the signal as being selected to excite different fragments, as detailed below, and the basic scheme is described in relation to the third mass selection step MS^3 , involving two fragmentation steps, with the control unit 24.

30 Each ion has a secular frequency ν , which is related to the drive frequency $\Omega/2\pi$, and the following Mathieu parameter β , as follows:

- 12 -

$$v = \frac{\beta\Omega}{2} \quad \text{for } n=0 \quad (1)$$

$$\text{For } q < 0.6, \text{ this reduces to } v \approx \left(a + \frac{q^2}{2}\right)^{\frac{1}{2}} \frac{\Omega}{2} \quad (2)$$

where a and q are standard Mathieu parameters given by:

$$a = \frac{8eU}{mr^2\Omega} \quad (3)$$

$$5 \quad q = \frac{4eV}{mr^2\Omega^2} \quad (4)$$

Thus, for $a = 0$, the relationship reduces to:

$$v = \frac{q\Omega}{2\sqrt{2}} \quad (5)$$

In accordance with the present invention, an excitation voltage is applied to the rod set Q2 at a frequency which is twice the secular
10 frequency, i.e. with a frequency of $\omega=2v$ ion. This would be at a potential v , in the range of 0.5 to 20 volts. This potential will be added to each of the potentials supplied to each pair of rods of the rod set Q2. Thus, the potential supplied to the pairs of rods would be as follows:

$$\begin{aligned} & V \cos \Omega t + v \cos (\omega t + \phi) \\ & - V \cos \Omega t - v \cos (\omega t + \phi) \end{aligned} \quad (6)$$

15 where ϕ is simply a factor to allow for the fact that the two signals need not necessarily be in phase.

Thus, to effect the different steps of MS³ and MS⁴, it is a matter of selecting different frequencies of ω , corresponding to ions of interest, as explained in greater detail in relation to the examples below. Alternatively,
20 however, from equation (5) it is evident that one could select a different RF voltage, or select a different q for a constant excitation frequency.

Then, in use, when modulation is effected by either of the units 26, 30, and note that this is irrespective of any voltage set by the power supply 24, 28, the output from the detector 20 is switched by the unit 32 30 alternately between the two data storage devices 34, 36, in synchronism with the modulation. This enables collection of two sets of data, one when

- 14 -

excitation is effected and one when excitation is not effected. As detailed below, this gives different spectra, which can be subtracted from one another.

Still referring to Figure 1, significantly, the use of a pre-selecting mass filter in the present application allows the first fragmentation to be non-selective, via a potential gradient. This is preferable because typically much more energy can be deposited into the initial ions, which may include hard to break bonds and massive molecules. The present inventors have appreciated that there would be sufficient cooling of the primary fragment ions and any residual precursor ions in the second multipole to permit efficient radial excitation for subsequent MS steps.

Reference will now be made to Figure 2. This shows an apparatus indicated generally by the reference 40. The apparatus 40 is similar to the apparatus 10, and for simplicity and brevity, like components are given the same reference numeral and the description of these components is not repeated. In brief, the apparatus 40 includes the first three quadrupole rod sets Q0, Q1 and Q2, and associated control and power supply elements.

However, here, to replace the final quadrupole Q3 on detector 20, there is provided a time-of-flight (TOF) mass analyzer 42. In known manner, the TOF analyzer of section 42 includes a gating region 44 and a detector 46. Thus, in use, ions pass into the gating region 44 and are gated or pulsed out to travel down the main body of the TOF 42, following a drift tube, until detected at a detector 46.

It will be appreciated that any suitable form of TOF could be provided. Thus, the TOF could comprise a reflectron or the like.

Reference will now be made to Figures 3-6 and also to Tables 1 and 2, which show mass spectra data collected in accordance with the present invention. All this data was collected on an apparatus using a TOF section, as in Figure 2.

Referring first to Figure 3a, there is shown a mass spectrum resulting from carrying out the first two MS steps, MS¹ and MS², on bosentan, a low mass chemical or drug, with a mass of 580. Thus, in Q1, the

- 15 -

voltages are set to select m/z 580 from bosentan, which is then accelerated into Q2 to fragment it, to generate the spectrum shown in Figure 3a; it will again be appreciated that the second mass analyzing step is in fact effected in TOF mass analyzer 42. As shown, this includes some residual amount of
5 the original bosentan at mass 580 and other significant peaks of fragments at 508 fragments close to mass 200 and others.

Figures 3b-3d then show subtracted spectra obtained by applying the third MS step, MS^3 , with a frequency set to excite an ion with an m/z 508, 202 and 280, respectively. For example, fragmentation of m/z
10 508 is achieved by applying a 4.5 volt excitation signal at a frequency of 220 kHz. As indicated on Figure 3b, this effects $MS/MS/MS$ (or MS^3).

Figure 3b shows a subtracted spectrum. Thus, Figure 3b shows the spectrum obtained by effecting the triple MS technique, with the spectrum of Figure 3a subtracted. Here, any negative quantities are shown
15 as zero. For example, the peak for mass 508 will, clearly, be much less in Figure 3b, so the subtraction of the spectrum of Figure 3a would give a negative value; in Figure 3b, this is graphed. This technique has the effect of subtracting any fragments that were present as a result of the MS^2 ion fragmentation. However, as explained below, further analysis of Figures 3b-
20 3d is required to determine which peaks are true MS^3 and which peaks result from incompletely subtracted spectra, due to signal fluctuations alone.

Figure 3e shows a scan obtained by effecting modulation with modulation control unit 26, to provide the received signal into the two
25 separate data streams, to collect two sets of data. However, the voltage supplied by the unit 24 is set to zero. In effect, Figure 3e shows the subtraction of what in theory should be two identical outputs. As can be seen, the spectra does show some measurable peaks. Note that these peaks result from, in effect, the subtraction of two relatively large quantities, to
30 give a small difference. The vertical scale in Figure 3e is different from that in the other figures. What this shows is that there will, in practice, be some fluctuation of the signal, and this can be some measure of the fluctuation

- 16 -

for individual fragments, and it can be noted that the fragment 202 shows a significant fluctuation. Thus, a statistical analysis of the significance level of the subtracted ion signal is used, as explained below. Processing of data sets collected for the statistical analysis allows identification and possible
5 elimination of the non-coherent variations in the ion signal.

While the statistical analysis is presented here by way of equations and tables, it will be appreciated that the analysis may be automated by implementing it in a software program running on a data processor, so as to process the data as it is recorded. This will permit rapid
10 "real time" determination of whether the value of the subtracted data is significant. Optionally, only data which is determined to be significant after such statistical analysis can be selected for presentation.

Turning to Figures 4a and 4b, these show test results and spectra obtained for the drug taxol. Figure 4a shows a basic two-step MS²
15 process. That is, taxol was selected in Q1, for transmission into Q2; the taxol is then accelerated into Q2 with a suitable potential difference, to cause CID or fragmentation of the taxol in Q2. The spectra in Figure 4a was then obtained.

Figure 4b then shows the spectrum obtained by further
20 excitation, i.e. MS³. Figure 4b is a subtracted spectrum. This shows a significant range of fragments for approximately 100 m/z to 400 m/z. Notably, even though there are significant peaks in this range in Figure 4a, the same ions are also generated by the subsequent fragmentation.

Figure 4c again shows a subtraction spectrum obtained
25 without any excitation. In other words, with modulation unit 26 actuated, to cause the data to be divided into two sets of data, but with the power supply 24, set to give zero excitation. Surprisingly, for taxol, this shows a significant residual background.

Referring now to Figures 5a and 5b these show, in greater
30 detail, a graphical representation of the signal obtained around the peak 124 and 122, as a result of exciting the fragment 202; thus these figures show details of the scan of Figure 3c.

- 17 -

Figure 5a shows two peaks 50 and 51. Peak 50 is the signal obtained with the additional excitation provided by the unit 24 turned off, and this also shows error bars indicating the variance in the signal obtained. Peak 51 shows the signal obtained with power supply 24 actuated, to provide
5 excitation of fragment 202, generating an additional quantity of the ion around mass 124. A subtracted spectrum would effectively show peak 51 minus peak 50. This demonstrates that a fragmentation of ion 202 does add significantly to a fragment at mass 124.

Figure 5b shows similar peaks 52 and 53 at mass 122. Again,
10 error bars for the peak 52 are shown. Peak 52 shows the spectra with no excitation of ion 202, while peak 53 shows the spectra with 202 excited. This shows where the two peaks are effectively identical, allowing for a margin of error. In other words, fragmentation of ion 202 does not add significantly to the signal at mass 122.

15 Thus, in order to ascertain which fragment signals are significant, the present invention incorporates statistical analysis for determining when fragmentation of a particular ion has added to the signal for a smaller fragment, and when no such effect is present. This is based on two basic principles, namely: firstly, simply subtracting the two peaks, as
20 indicated for the peaks in Figures 5a, 5b and determining that there is a significant additional added signal, when there is a significant and measurable difference between the two peaks; and comparing two peaks to determine if there is significant fluctuation in values. This latter feature is explained in greater detail in relation to Tables 1 and 2.

25 Referring first to Table 1, this shows four sets of data, for different peaks at, approximately 124, 98, 106 and 79, where it is determined that fragmentation of the 202 ion did add significantly to a peak. These peaks were chosen, representative of, respectively, "medium", "little", "big" and very little peaks, the adjectives indicating relative peak size. For each
30 ion, there are two columns, indicating the count made, with excitation on and excitation off respectively.

Thus, for ion 124, counts are obtained at masses ranging from

124.0131 to 124.0735. The final column calculates a significance factor $|T|$ or "Sig" using a statistical method called the "T" test. This test permits comparison of two parent populations to determine the degree to which they are different. This method is derived from probability statistics assuming a Gaussian distribution of ions in time; other probability functions may be used. While this statistical method considers the magnitude of the significance factor, other methods may take polarity information into consideration. The value of $|T|$ is calculated by the following equation:

$$|T| = \text{Sig} = \left| \frac{\text{detected ion signal, alternating current on} - \text{detected ion signal, alternating current off}}{\sqrt{\sigma^2 \text{ alternating current on} + \sigma^2 \text{ alternating current off}}} \right| \quad (7)$$

where σ is the standard deviation. Here, a value of $|T|$ of \sim two or less, indicates that there is a greater than \sim 95% probability that the excitation on and off signals are the same. On the other hand, for this mass 124, one can see that the values of $|T|$, at the peak, are in excess of 10, clearly indicative of a substantial difference, and this is borne out by the visual representation in Figure 4a.

Similar results, although not quite so strong, were obtained for the peak and mass 98. This again shows that, for nearly all values around the peak 98, the on signal gave a higher signal than the off signal. Again, value of $|T|$ was quite high around the peak.

In general, it would be noted that it is more difficult to make a clear determination for smaller peaks.

For a large or big peak, as shown for the mass 106, the difference between the on and off signals was significant, and it is noted that the value of $|T|$ reached a value of in excess of 57 close to the peak. This is clearly indicative of a substantial difference between the on and off signals, thereby indicating that the fragmentation of ion 202 did contribute significantly to the fragment and mass 106.

Finally, for the ion at mass 79, this represents another, smaller peak. This again gives a clear indication that there was a difference between the two signals.

TABLE 1

<u>M/Z</u>	<u>ON</u>	<u>OFF</u>	<u> T </u>
		<u>MED#, YES</u>	
<u>124.0131</u>	<u>7</u>	<u>11</u>	<u>0.943</u>
<u>124.0186</u>	<u>20</u>	<u>11</u>	<u>1.62</u>
<u>124.0241</u>	<u>40</u>	<u>36</u>	<u>0.459</u>
<u>124.0296</u>	<u>162</u>	<u>149</u>	<u>0.737</u>
<u>124.0351</u>	<u>1117</u>	<u>874</u>	<u>5.44</u>
<u>124.0406</u>	<u>3854</u>	<u>3036</u>	<u>9.85</u>
<u>124.0461</u>	<u>6377</u>	<u>4865</u>	<u>14.3</u>
<u>124.0516</u>	<u>5321</u>	<u>4073</u>	<u>12.9</u>
<u>124.0571</u>	<u>2596</u>	<u>2164</u>	<u>6.26</u>
<u>124.0626</u>	<u>1420</u>	<u>1163</u>	<u>5.06</u>
<u>124.0681</u>	<u>1016</u>	<u>829</u>	<u>4.35</u>
<u>124.0735</u>	<u>663</u>	<u>566</u>	<u>2.77</u>
		<u>LITTLE, YES</u>	
<u>98.0192</u>	<u>1</u>	<u>1</u>	<u>0</u>
<u>98.0241</u>	<u>4</u>	<u>2</u>	<u>0.816</u>
<u>98.0289</u>	<u>13</u>	<u>13</u>	<u>0</u>

	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100	2101	2102	2103	2104	2105	2106	2107	2108	2109	2110	2111	2112	2113	2114	2115	2116	2117	2118	2119	2120	2121	2122	2123	2124	2125	2126	2127	2128	2129	2130	2131	2132	2133	2134	2135	2136	2137	2138	2139	2140	2141	2142	2143	2144	2145	2146	2147	2148	2149	2150	2151	2152	2153	2154	2155	2156	2157	2158	2159	2160	2161	2162	2163	2164	2165	2166	2167	2168	2169	2170	2171	2172	2173	2174	2175	2176	2177	2178	2179	2180	2181	2182	2183	2184	2185	2186	2187	2188	2189	2190	2191	2192	2193	2194	2195	2196	2197	2198	2199	2200	2201	2202	2203	2204	2205	2206	2207	2208	2209	2210	2211	2212	2213	2214	2215	2216	2217	2218	2219	2220	2221	2222	2223	2224	2225	2226	2227	2228	2229	2230	2231	2232	2233	2234	2235	2236	2237	2238	2239	2240	2241	2242	2243	2244	2245	2246	2247	2248	2249	2250	2251	2252	2253	2254	2255	2256	2257	2258	2259	2260	2261	2262	2263	2264	2265	2266	2267	2268	2269	2270	2271	2272	2273	2274	2275	2276	2277	2278	2279	2280	2281	2282	2283	2284	2285	2286	2287	2288	2289	2290	2291	2292	2293	2294	2295	2296	2297	2298	2299	2300	2301	2302	2303	2304	2305	2306	2307	2308	2309	2310	2311	2312	2313	2314	2315	2316	2317	2318	2319	2320	2321	2322	2323	2324	2325	2326	2327	2328	2329	2330	2331	2332	2333	2334	2335	2336	2337	2338	2339	2340	2341	2342	2343	2344	2345	2346	2347	2348	2349	2350	2351	2352	2353	2354	2355	2356	2357	2358	2359	2360	2361	2362	2363	2364	2365	2366	2367	2368	2369	2370	2371	2372	2373	2374	2375	2376	2377	2378	2379	2380	2381	2382	2383	2384	2385	2386	2387	2388	2389	2390	2391	2392	2393	2394	2395	2396	2397	2398	2399	2400	2401	2402	2403	2404	2405	2406	2407	2408	2409	2410	2411	2412	2413	2414	2415	2416	2417	2418	2419	2420	2421	2422	2423	2424	2425	2426	2427	2428	2429	2430	2431	2432	2433	2434	2435	2436	2437	2438	2439	2440	2441	2442	2
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- 22 -

M/Z	ON	OFF	T
79.073	3	0	1.73

Turning to Table 2, this shows sets of data indicating a situation where fragmentation of ion 202 showed little variation in the on and off signals, indicating that the peaks were essentially the same, and for which the additional third MS step added nothing to the peak. Table 2 again shows, in the same order, data for a medium, little, big and very little peaks, at masses 122, 131, 123 and 103 respectively.

The column for the factor $|T|$ shows that for the mass 122, $|T|$ often has a value of much less than 1, and only exceeds 1 for a couple of the data points. This is clearly indicative of two peaks that are the same and have no statistically different magnitude. This data corresponds to Figure 4b.

There is a similar effect for a small or little peak for the mass 131. Here, the values of $|T|$ are even smaller, and it can be seen that many of the values for the difference figure are negative or very small.

For a big peak at mass 123, due to the larger size of the peaks, values for the difference and significance parameter $|T|$ are larger. Here, a review of the various values of the parameter $|T|$ again clearly shows that these two peaks are substantially the same.

Finally, for mass 103, it can be noted that the values for the difference in $|T|$ data are all extremely small. Again, a clear indication that there is no statistically significant difference between the two peaks.

TABLE 2

M/Z	ON	OFF	T
	MED#,NO		

M/Z	ON	OFF	T
122.0154	12	9	0.655
122.0208	27	31	0.525
122.0263	76	92	1.23
122.0318	170	162	0.439
122.0372	153	159	0.340
122.0427	364	411	1.699
122.0481	1192	1289	1.95
122.0536	2480	2365	1.65
122.059	2381	2496	1.65
122.0645	1325	1401	1.46
122.0699	622	596	0.745
122.0754	285	257	1.20
122.0808	159	170	0.606
	LITTLE,NO		
131.017	1	2	0.577
131.0226	12	12	0.000
131.0282	18	20	0.324
131.0339	26	22	0.577
131.0395	32	49	1.89
131.0452	132	133	0.061
131.0508	324	311	0.516

- 24 -

M/Z	ON	OFF	T
131.0565	463	507	1.41
131.0621	335	333	0.077
131.0678	172	186	0.740
131.0734	212	226	0.699
131.0791	385	386	0.036
131.0847	405	391	0.496
131.0904	204	203	0.050
131.096	81	86	0.387
		BIG,NO	
123.0259	220	263	1.957
123.0314	1108	1098	0.213
123.0368	2737	2943	2.73
123.0423	3539	3554	0.178
123.0478	2622	2738	1.58
123.0533	3409	3343	0.803
123.0587	7021	7081	0.505
123.0642	8916	8623	2.21
123.0697	5861	5698	1.52
123.0752	2345	2247	1.45
123.0806	957	945	0.229
123.0861	585	587	0.058

- 25 -

M/Z	ON	OFF	T
	V.LITTLE,NO		
103.0308	4	9	1.39
103.0358	10	10	0
103.0408	38	37	0.115
103.0458	79	85	0.469
103.0508	140	146	0.355
103.0558	103	112	0.614
103.0608	46	47	0.104
103.0658	8	22	1.96
103.0708	14	15	0.186
103.0758	6	3	1.0
103.0809	2	2	0
103.0859	4	2	0.816
103.0909	5	4	0.333
103.0959	2	3	0.447
103.1009	2	4	0.816
103.1059	0	0	0

Referring now to Figures 6a, 6b and 6c, these show further spectra obtained for reserpine. Figure 6a again shows just the first two MS steps, where reserpine is selected in Q1, accelerated and fragmented in Q2.

- 26 -

Additionally, here Figure 6a just shows the low mass end of the fragment spectrum up to approximately mass 200. This shows that reserpine with an m/z of 609 generates significant fragments at 174.1 and 195.1.

Figure 6b then shows the spectrum obtain by a third MS step, where the fragment at 174 was excited. As might be expected, this shows a much reduced peak for the mass 174, and an increase in the number and intensity of fragments below mass 174, notably peaks at 130.1 and 131.1. Unlike earlier figures, Figure 6b is an unsubtracted spectrum.

If the spectrum of Figure 6a is subtracted from Figure 6b, the spectra of Figure 6c is obtained. Note that this is on a different scale. This clearly shows a significant reduction in the peak at 195.1, as this was present in the original spectrum of Figure 6a. This spectrum also emphasizes the contribution made to the various other fragments by the third MS step, the major peaks being identified in Figure 6c.

Reference will now be made to Figures 7a, 7b and 7c. Figure 7a shows part of the spectrum of Figure 6a but only up to a mass of approximately 190. This enables a different scale to be used, to emphasize the size of the different peaks.

Figure 7b then shows a spectrum obtained for a four-step excitation scheme. Here, the fourth MS step, MS^4 was effected utilizing the power supply 28 and modulation unit 30. For this scheme, the excitation as a third MS step, by the power supply 24, is continuous, without any modulation by the unit 26. The spectrum obtained is then subject to further excitation of the mass at 130/131; these two masses are so close together, that it is impossible to obtain excitation of just one mass. Again, Figure 7b is an unsubtracted spectrum.

Figure 7c then shows the spectrum of Figure 7b, with that of Figure 7a subtracted. This again, shows elimination of peaks due to previous fragmentation and hence solely the peaks resulting from ions generated by fragmentation of the ions of mass 130, 131. It should be noted that for the fourth step MS^4 procedure, excitation from the two power supplies 24, 28 is provided simultaneously. As noted, the power supply 24

- 27 -

is unmodulated, i.e. continuous, while the excitation from power supply 28 is modulated at a modulation of, for example, 2 Hz.

Reference will now be made to Figures 8a-8d, which show a series of spectra, indicating the effects of varying the excitation voltage.

- 5 Figure 8a again corresponds to Figure 6a, and shows the fragment spectrum obtained from the initial fragmentation of the Reserpine, again showing significant peaks at 174.1 and 195.1. In this case, the larger peak at 195.1 was selected for further excitation. This was excited at a frequency of 575 kHz and at different voltages of 1.5, 2.5 and 3.5, to obtain the spectra of
- 10 Figures 8b, 8c and 8d. Each of these spectra 8b-8d are subtracted spectra, that is the spectra obtained with the excitation and subsequent subtraction of the spectrum of Figure 8a. They are also unfiltered.

- As might be expected, the peak at 195 is largely eliminated as a result of the excitation. It can be noted that at low excitation potentials, a
- 15 peak is shown with an ion close to mass 190, and this peak reduces significantly, as the excitation voltage is increased. Correspondingly, peaks with smaller fragment ions increase. This is to be expected.

- It will be appreciated that, while the invention has been described as effected with a quadrupole, it can be carried out in any suitable
- 20 collision cell, and in particular any collision cell where quadrupolar fields can be applied. Also, while using a quadrupole is preferable, it will be appreciated that other multipolar guides may be used.

- 28 -

CLAIMS:

1. A method of analyzing a substance, the method consisting of:
- (1) creating a stream of ions in said substance;
 - (2) supplying the stream of ions and a collision gas to a
 - 5 multipole and providing an RF signal to the multipole, whereby the quadrupole functions as a collision cell;
 - (3) fragmenting said ions in the RF multipole by collisions with the gas molecules, in order to form primary fragment ions;
 - (4) supplying additional alternating current to the
 - 10 multipole at a frequency selected to cause resonance excitation of a desired primary fragment ion mass-to-charge ratio, whereby ions with said desired primary fragment ion mass to charge ratio are excited and undergo collisions with the gas molecules causing production of secondary fragment ions;
 - (5) modulating the alternating current signal applied in step
 - 15 (4) whereby periods in which said alternating current signal is applied alternate with periods in which the alternating signal is not applied;
 - (6) detecting the ion signal after fragmentation with a mass spectrometer and collecting one set of data for one spectrum,
 - 20 representative of the ion spectrum when the alternating current signal is applied and another set of data for another spectrum, representative of the ion spectrum when the alternating current signal is not applied;
 - whereby said other spectrum can be subtracted from said one spectrum, to generate a subtracted spectrum showing the secondary fragment ions
 - 25 without the presence of the primary fragment ions except for any said primary fragment ions which are generated by step (4), whereby to obtain MS³ information.
2. A method as claimed in claim 1, further including the step of processing the data sets by applying statistical analysis to reject spectra
- 30 having statistically insignificant variations in the ion signal.

- 29 -

3. The method as claimed in claim 2, wherein the statistical analysis is implemented in a software program and performed automatically.
4. The method as claimed in claim 3, wherein the statistical analysis is performed in real time so that spectra having statistically insignificant variations in the ion signal are not displayed.
5. A method as claimed in claim 1, wherein, said multipole is a quadrupole.
6. A method as claimed in claim 5, which includes applying the alternating current signal at a frequency that excites the desired primary fragment ion.
7. A method as claimed in claim 5, which includes passing the stream of ions through a first mass analyzer to select a precursor ion mass to charge ratio of interest, and passing the precursor ions into the collision cell.
8. A method as claimed in claim 7, which includes providing a potential difference between the first mass analyzer and the collision cell, to accelerate the precursor ions into the collision cell, whereby the precursor ions gain sufficient velocity to collide with the collision gas to cause fragmentation, and wherein step (4) comprises applying an alternating current signal to excite the desired primary fragment ions.
9. A method as claimed in claim 7 or 8, which includes applying a second alternating current signal to the quadrupole rod set, to excite the secondary fragment ions generated in step (4), thereby to generate tertiary fragment ions and wherein step (5) comprises modulating the second

14. An apparatus, for analyzing a substance by resonance
excitation of selected ions and selective collision-induced dissociation, the
25 apparatus comprising:

a collision cell, including a quadrupole ion guide, for receiving a stream of precursor ions and provided with a collision gas, for collision-induced dissociation between the parent ions and the buffer gas;

a modulation means connected to the power supply, for modulating the alternating current signal, whereby periods in which said alternating current signal are applied alternate with periods in which the alternating current signal is not applied.

16. An apparatus as claimed in claim 14, which includes a second power supply connected to the quadrupole rod set, a second modulation unit connected to the second power supply and also to the switch, before applying a second alternating current signal, for excitation of a second ion.

18. An apparatus as claimed in claim 16, which includes a final mass analysis section, including the detector, for analyzing fragment ions

- 32 -

from the collision cell.

19. An apparatus as claimed in claim 17, wherein the final mass analysis section comprises one of:

a scanning mass analyzer and a detector; and

5 a time-of-flight device, including the detector for providing a small spectrum.

[illegible]

ABSTRACT OF THE DISCLOSURE

A method of and apparatus for analyzing a substance takes a stream of ions in said substance and supplies the ions to a collision cell including a quadrupole rod set for guiding the ions and a buffer gas. An RF voltage is applied to the quadrupole rod set to guide ions. An additional alternating current signal is applied to the quadrupole rod set at a frequency selected to cause resonance excitation of the secular frequency of a desired ion, whereby said desired ions are excited and undergo collision with the buffer gas causing fragmentation. The invention then provides modulation of the alternating current signal applied whereby periods in which said alternating current signal is applied alternate with periods in which said alternating signal is not applied. The ion spectrum after fragmentation is collected to generate one set of data for one spectrum, representative of the ion spectrum when the alternating current signal is applied, and a another set of data for another spectrum, representative of the ion spectrum when the alternating current signal is not applied. These two spectra can be subtracted to give a spectrum indicative of the effect of fragmentation.

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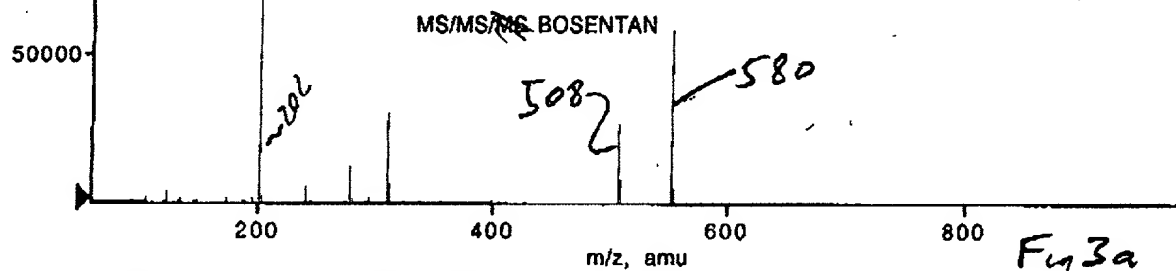
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Friday, May 21, 1999 5:42 PM

page 1 of 1

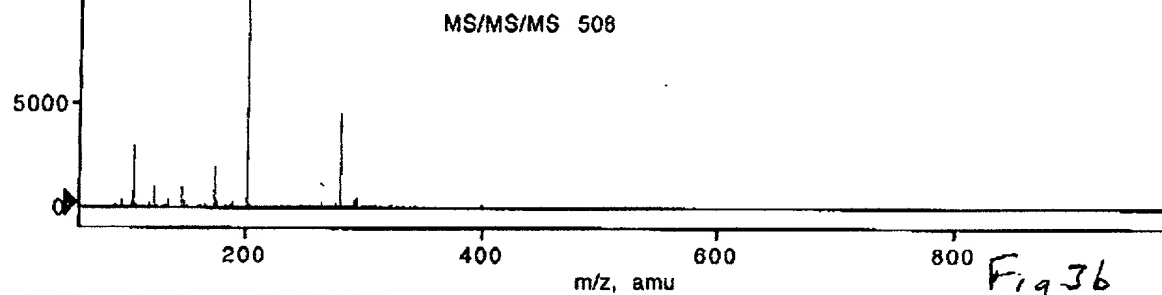
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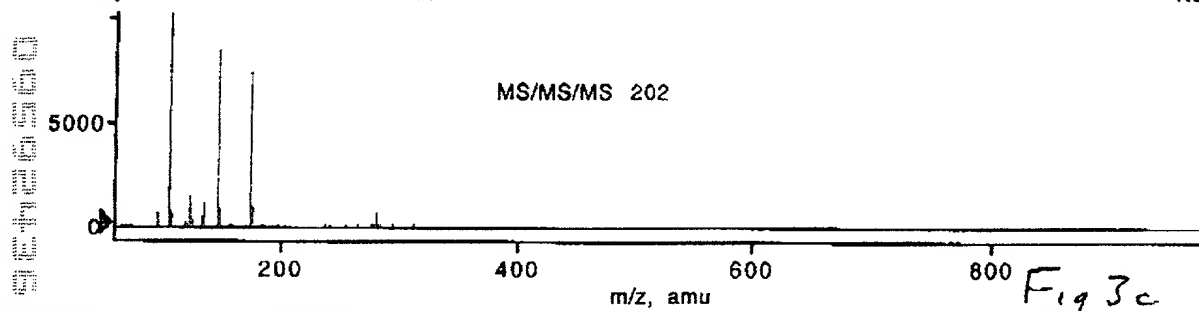
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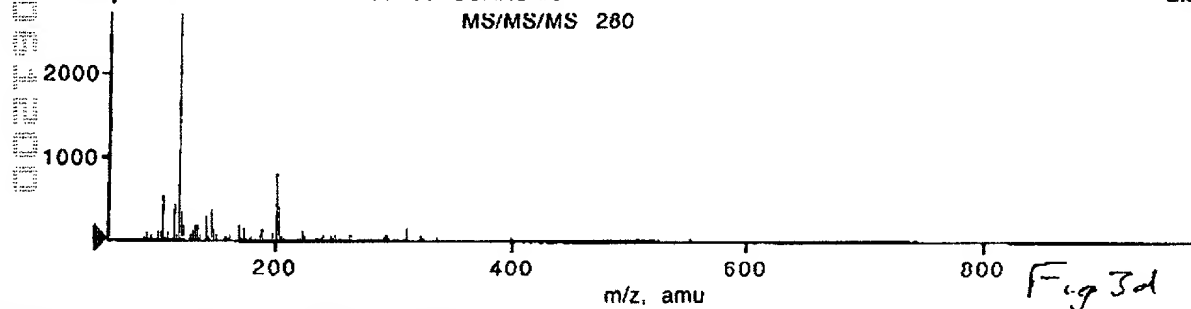
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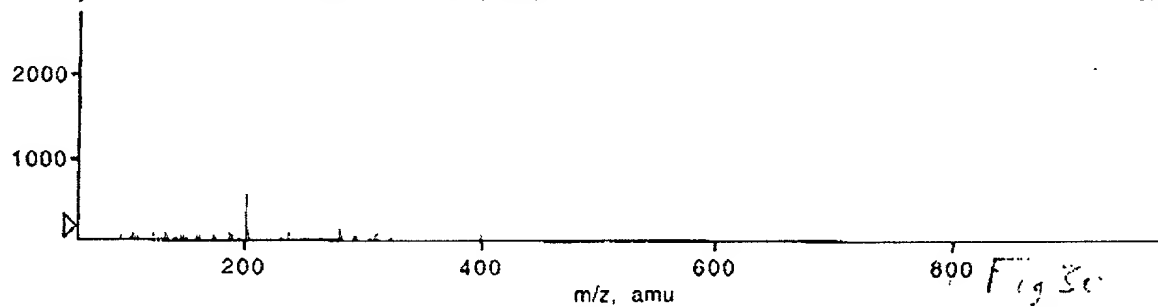
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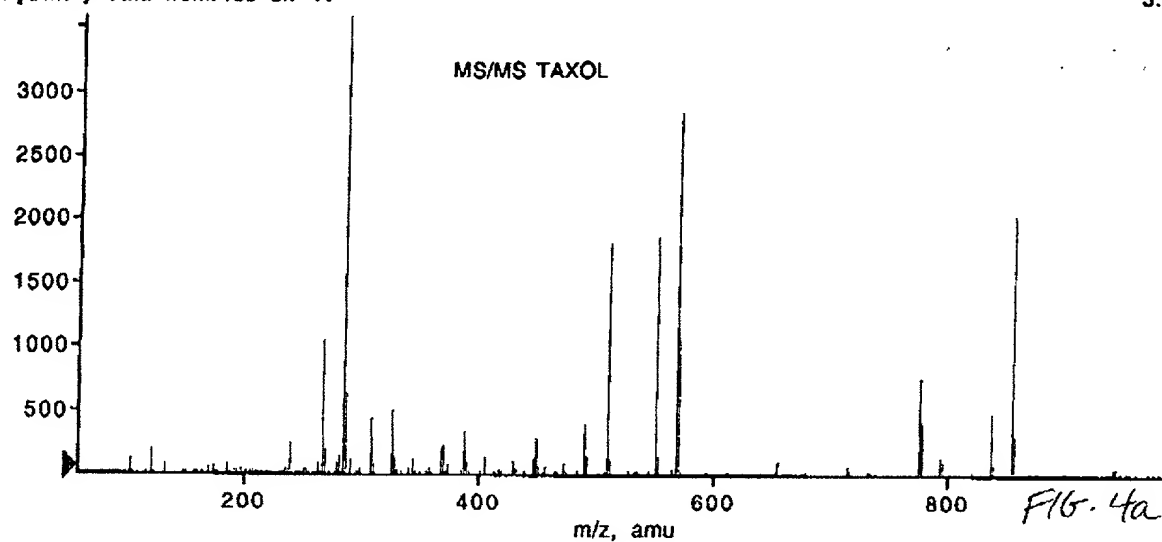
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page 1 of 1

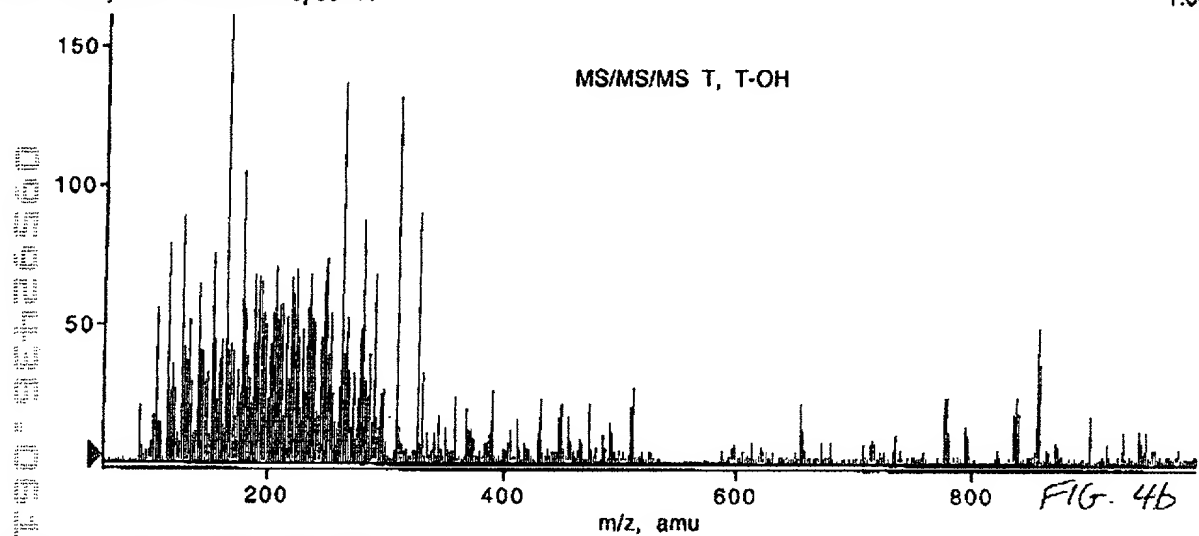
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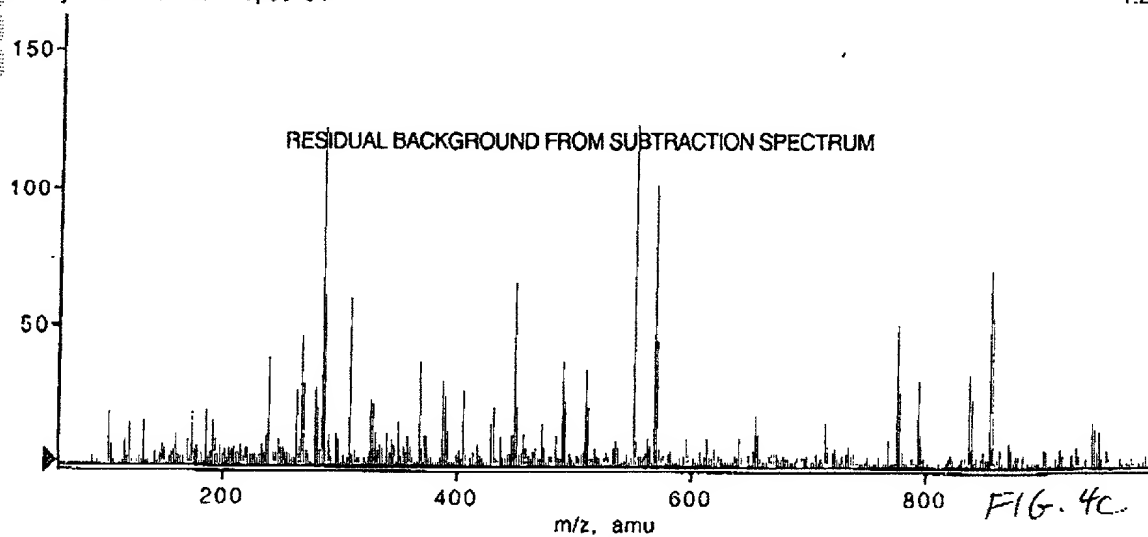
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Sheet1

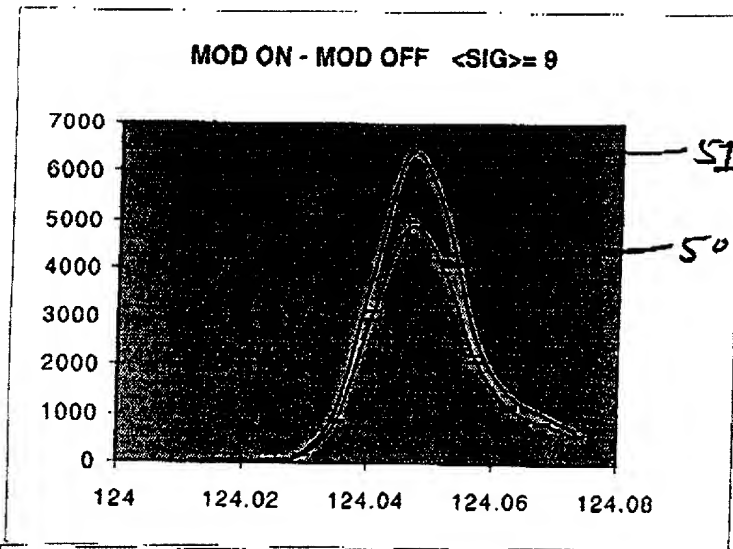


FIG. 5a

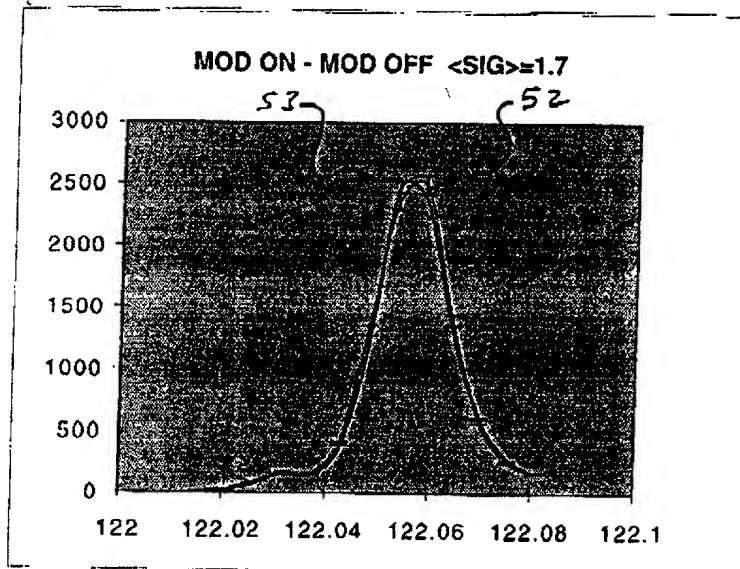
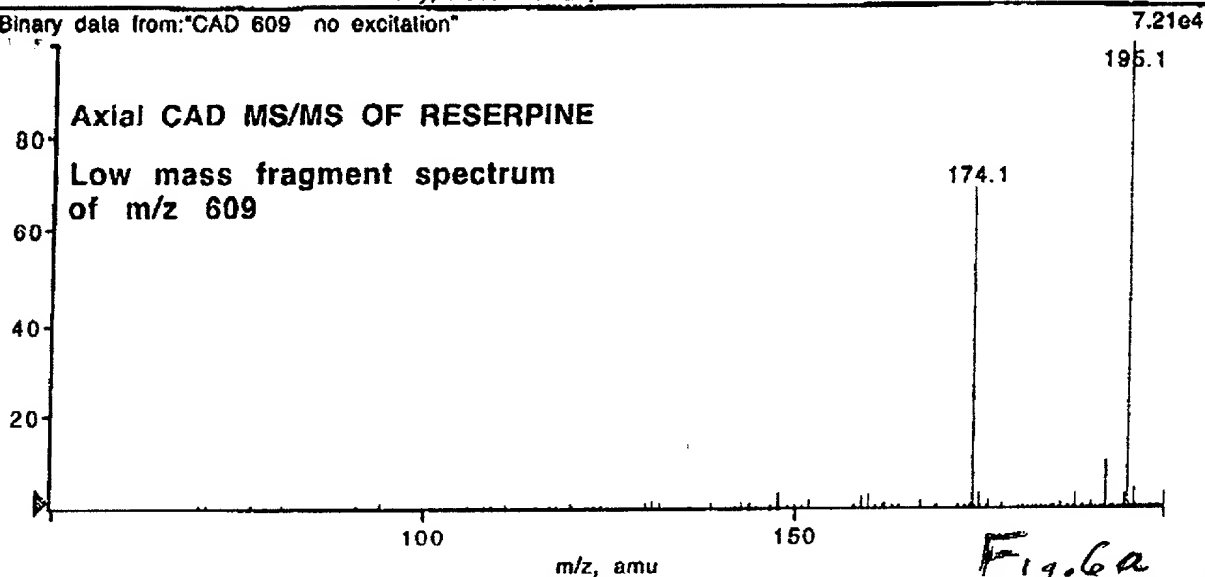
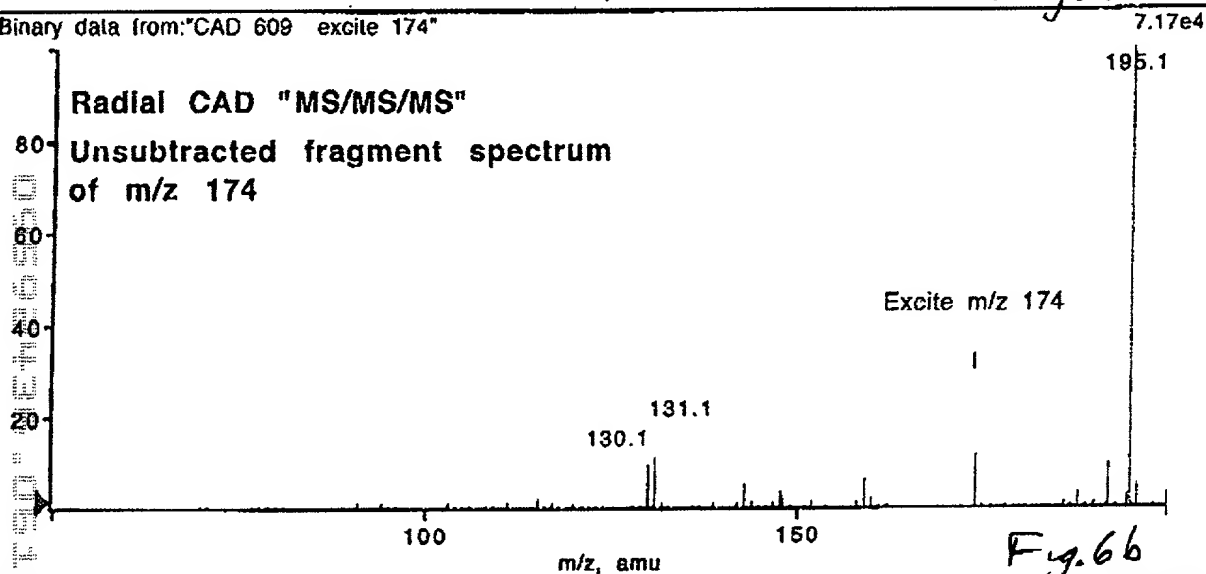


FIG. 5b

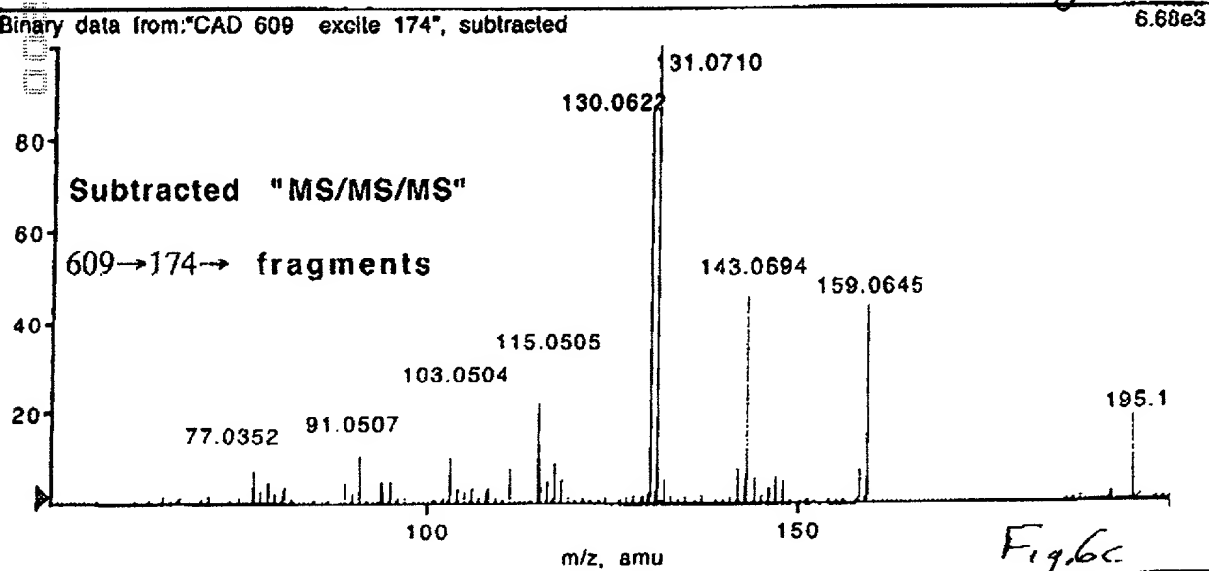
OF Binary data from:"CAD 609 no excitation"



F Binary data from:"CAD 609 excite 174"



Binary data from:"CAD 609 excite 174", subtracted



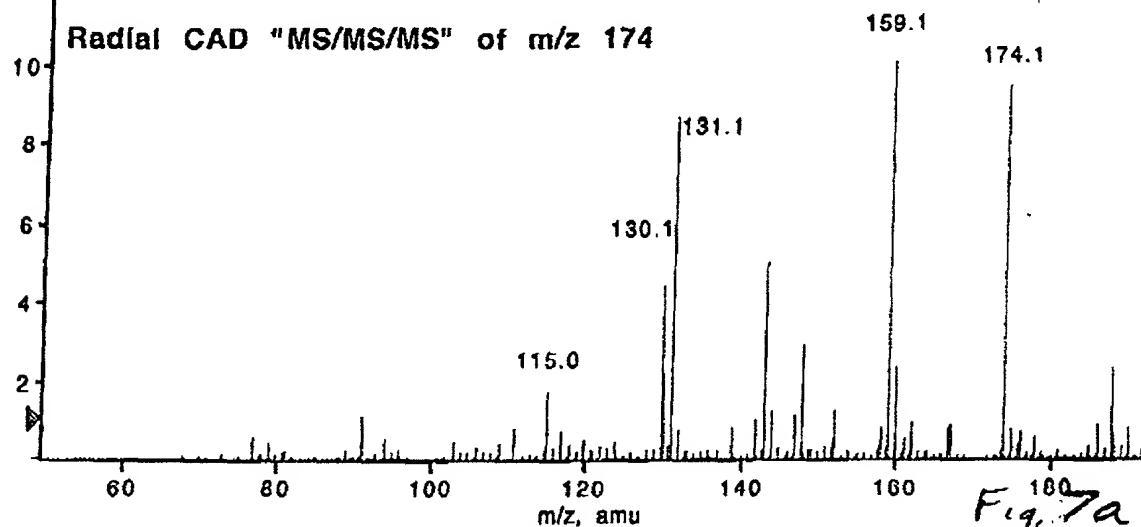
ultraView 1.5d0

Friday, November 27, 1998 5:49 PM

page 1 of 1

Binary data from: "CAD 609, excite 174 only"

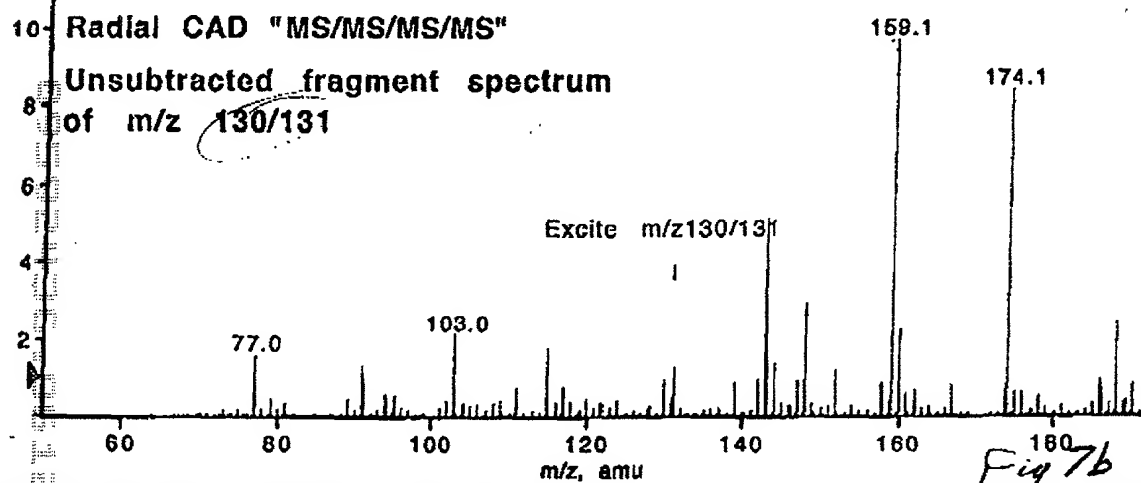
2.07e4



Binary data from: "CAD 609, excite 174 and 131"

2.13e4

Radial CAD "MS/MS/MS/MS"

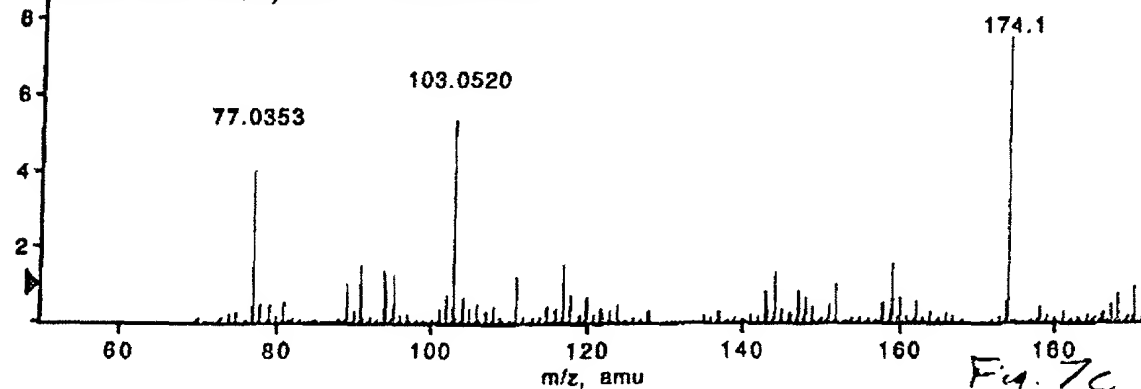
Unsubtracted fragment spectrum
of m/z 130/131

Binary data from: "CAD 609, excite 174 and 131", subtracted

7.20e3

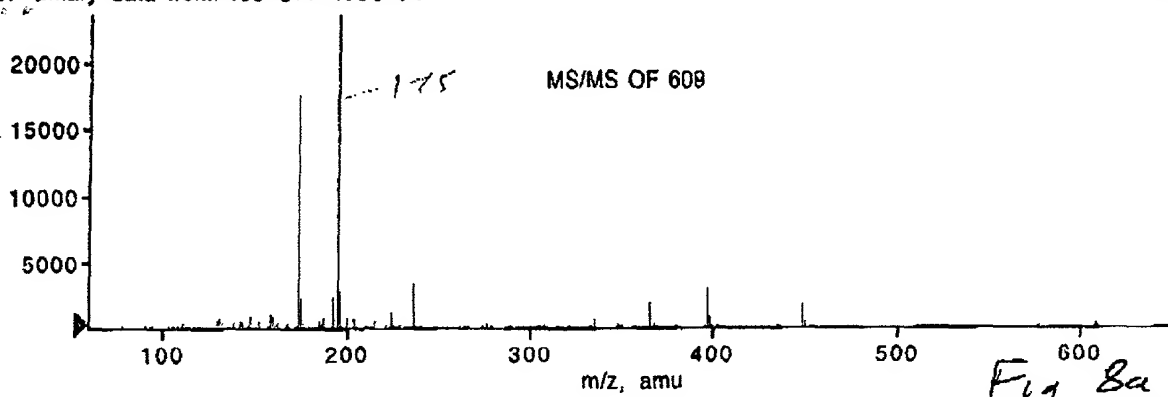
Subtracted "MS/MS/MS/MS"

609→174→130,131→ fragments



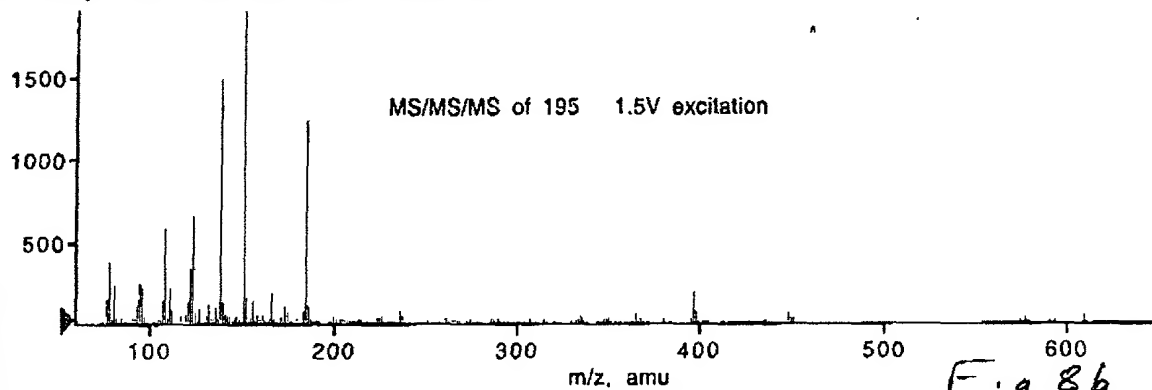
TOF Binary data from:"195 OFF-40SC-OV"

2.35e4



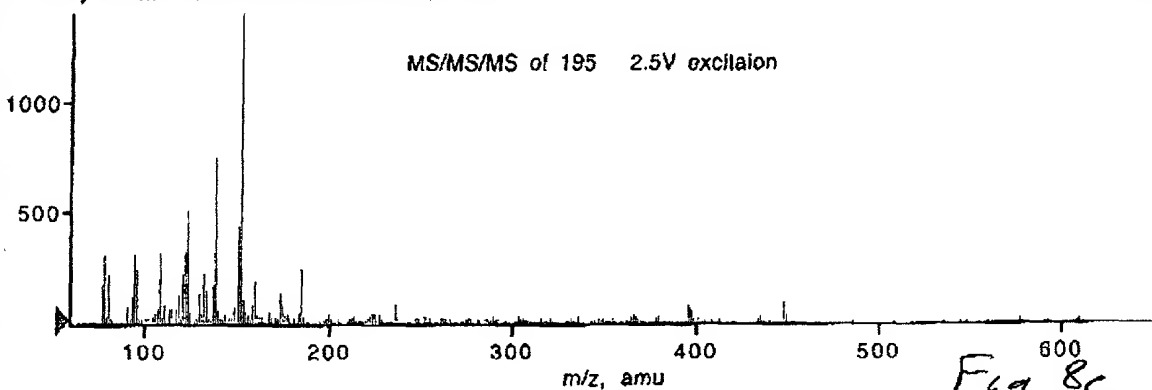
TOF Binary data from:"195--1.5V--1S-40SC--#2"

1.90e3



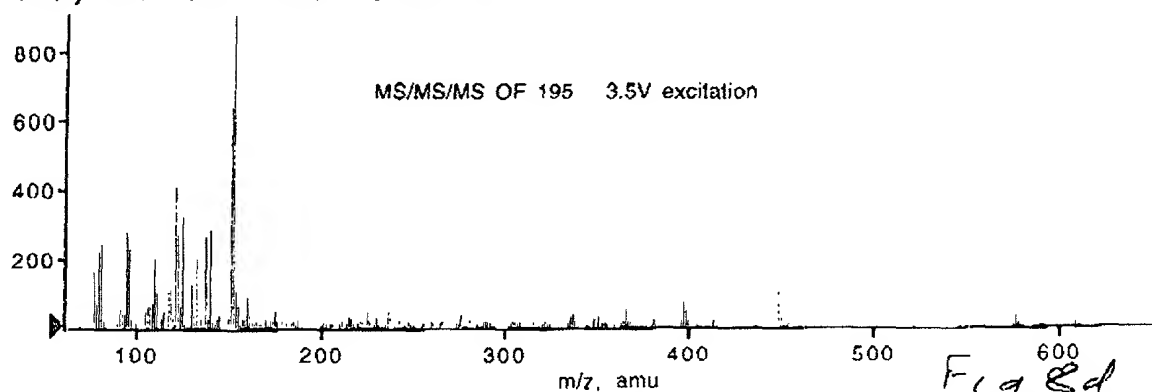
TOF Binary data from:"195--2.5V--1S-40SC--#2"

1.39e3



TOF Binary data from:"195--3.5V--1S-40SC--#2"

9.09e2



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PATENT APPLICATION
(37 CFR 1.63)**☐ Declaration
Submitted
with Initial
Filing
OR
☐ Declaration
Submitted after Initial
Filing (surcharge
(37 CFR 1.16 (e))
required)

Attorney Docket Number	571-651
First Named Inventor	LISA COUSINS
COMPLETE IF KNOWN	
Application Number	/
Filing Date	
Group Art Unit	
Examiner Name	

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

**ANALYSIS TECHNIQUE, INCORPORATING SELECTIVELY INDUCED COLLISION DISSOCIATION AND
SUBTRACTION OF SPECTRA**

the specification of which (Title of the invention)

☒ is attached hereto

OR

☐ was filed on (MM/DD/YYYY) as United States Application Number or PCT International

Application Number and was amended on (MM/DD/YYYY) (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT International application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached?	
				YES	NO
2,274,186	CANADA		<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SR/02N attached hereto.

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Application Number(s)	Filing Date (MM/DD/YYYY)	
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(Page 1 of 2)

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U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)

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OR

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Richard J. Parr	22,836	Philip Mendes da Costa	33,106
H. Roger Hart	26,426	Robert B. Storey	33,108
David W.R. Langton	27,747	John R. Rudolph	35,003
C. Lloyd Sarginson	29,245	Robin L.A. Coster	38,016
Timothy J. Sinnott	31,083	Michael E. Charles	38,036

☒ Additional registered practitioner(s) named on supplemental Registered Practitioner Information sheet PTO/SB/02C attached hereto.

Direct all correspondence to: ☒ Customer Number **1059** OR ☐ Correspondence address below

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that those statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of Sole or First Inventor: ☐ A petition has been filed for this unsigned inventor

Given Name (first and middle (if any))		Family Name or Surname	
Lisa		Cousins	
Inventor's Signature		Date	
Residence: City	Toronto	State	Ontario
		Country	Canada
		Citizenship	Canadian
Post Office Address	177 Keele Street		
Post Office Address			
City	Toronto	State	Ontario
		ZIP	M6P 2K1
		Country	Canada

☒ Additional inventors are being named on the ¹ supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto

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DECLARATION	ADDITIONAL INVENTOR(S) Supplemental Sheet Page <u>1</u> of <u>1</u>
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Name of Additional Joint Inventor, if any:		<input type="checkbox"/> A petition has been filed for this unsigned inventor					
Given Name (first and middle (if any))				Family Name or Surname			
Bruce				Thomson			
Inventor's Signature						Date	
Residence: City	Etobicoke	State	Ontario	Country	Canada	Citizenship	Canadian
Post Office Address	16 Orrell Avenue						
Post Office Address							
City	Etobicoke	State	Ontario	ZIP	M9A 1J9	Country	Canada
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Given Name (first and middle (if any))				Family Name or Surname			
Inventor's Signature						Date	
Residence: City		State		Country		Citizenship	
Post Office Address							
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